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CHROMOPLASTS—DEVELOPMENT OF CRYSTALLINE FORMS, STRUCTURE, STATE OF THE PIGMENTS

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I. INTRODUCTION

The present review is limited to a discussion of the shapes of chromoplasts—their development, their structure and the state of their pigments. Besides round forms, similar to those of chloroplasts, chromoplasts appear in a variety of other forms, such as filaments, spindles, rings, spirals, triangles, ribbons (tetraeders), pentaeders, hexaeders and bodies with concavely or convexly curved sides and drawn out points. Since Meyer's (1883*a*) discussion, it has been accepted by most investigators that the peculiar forms of chromoplasts are caused by crystallisation of the pigments. This erroneous opinion has prevented better understanding of the

development of chromoplasts and of the relationship between them and cytoplasm. The present literature review is concerned mostly with this aspect of the problem. It does not deal with the types of carotinoid pigments or with the tissues or plants in which they occur, nor with the function of chromoplasts and pigments, a problem of which not much is known.

Very different opinions have been expressed to explain the development and peculiar shapes of chromoplasts. The greatest awareness of the problem can be found in the early stages of research, from about 1860 to 1890, and the literature of those years is cited in this review more extensively than that of the present century. Afterwards not many new points of view appear, except regarding the relationship between chromoplasts and chondrioconts.

Some observations by the present writer concerning the development and the shapes of carrot chromoplasts are described later in this paper. These observations may facilitate a better appreciation of the problem in general, and, since they indicate a close relationship between chromoplasts and cytoplasm, data of the literature which point in the same direction are also reviewed.

II. THEORIES CONCERNING THE DEVELOPMENT OF PLASTIDS

Since all plastids (leucoplasts, chloroplasts, chromoplasts) can develop from one another, the same fundamental laws are probably valid for all of them. Before, therefore, considering chromoplasts, the theories found in the literature concerning the development of plastids in general will be briefly reviewed.

v. Mohl (1855), Sachs (1862), Hofmeister (1867), Strassburger (1876) and others later believed that plastids differentiate directly from cytoplasm. According to these investigators, areas of increased light reflection appear in the cytoplasm before the pigments are formed. In the first stage of differentiation, these areas often show polygonal outlines but later become round (v. Mohl, Sachs, Hofmeister). Schimper (1880) originally shared this opinion, but he changed it when Schmitz (1883) and he himself found differentiated plastids already in embryonic cells. Schimper (1883*a*) and Meyer (1883*b*) then concluded that plastids are never formed de novo but are derived by division from other plastids which pre-exist in embryonic cells and are transmitted through the germ cells to the next generation. This opinion was accepted by many investigators.

A different view about the origin of plastids was held by Pensa (1910), Lewitsky (1910), Guillermond (1912), and supported by Meves (1917), Cowdry (1918), Alvarado (1923) and others. According to these investigators, plastids derive from mitochondria, structures found in all plant and animal cells and having the forms of granules, rodlets or filaments. Most investigators believe that mitochondria are not formed de novo but pre-exist in embryonic cells and are transmitted through the germ cells to the next generation, and that all other mitochondria as well as plastids derive from them. According to Guillermond (1920), Emberger (1921) and Mangenot (1922), there exist two genetically different kinds of mitochondria, one kind which develops into plastids, and the other kind which does not. Reasons against the existence of two types of mitochondria were expressed recently by Newcomer (1946) who has also reviewed the present knowledge about mitochondria in plant cells (Newcomer, 1951).

The opinion that plastids derive from mitochondria was not shared by all investigators. Rudolph (1912), Scherrer (1913), Sapehin (1915), Mottier (1918), Dangeard (1919) and others thought that plastids and mitochondria are independent of each other. Their view is based mostly on observations of lower plants, especially algae, where the pastids of embryonic cells already contain pigments. In higher plants the embryonic and meristematic cells contain colorless granules which are considered as mitochondria by one group of investigators and as leucoplasts by the other. According to Alvarado (1923), it is no contradiction to assume that plastids derive as well by division from other plastids as by differentiation from mitochondria. Recently Weier and Stocking (1952) have criticised the identification of proplastids with mitochondria, and also the tendency to designate all cytoplasmic granules as mitochondria. De Rezende-Pinto (1952) also contested the derivation of plastids from mitochondria and described observations in favor of the differentiation of chloroplasts from cytoplasmic fibrils.

As was already mentioned, leucoplasts, chloroplasts and chromoplasts can develop from one another. The development of chromoplasts from leucoplasts and chloroplasts was described by Meyer (1883*b*) and Schimper (1885), and from mitochondria by Guillermond (1917, 1919). Very often chromoplasts represent final forms of metamorphosis of the other plastids. This is, however,

not always the case (Schimper, 1885), and chromoplasts can also undergo further transformation into chloroplasts or leucoplasts. Chromoplasts of this type were reported by Molisch (1902), Geitler (1937*a*) and especially by Rotherth (1912) who found this transformation in many tropical plants.

III. THEORIES RELATING TO THE CRYSTALLINE SHAPES OF CHROMOPLASTS

About 65 years ago several investigators made an intensive study of chromoplasts and discussed different possibilities to explain the crystal-like forms. Schimper (1883*a*) and Meyer (1883*a*) agreed that crystallisation must determine these forms, but they did not agree about what crystallised. Schimper believed that the crystalline forms are due to protein crystals (spindles, needles, ocataeders, etc.), the same that he had often observed in leucoplasts. He regarded these protein crystals as the protoplasm of the plastids in an inactive state which can become active again. Meyer (1883*a*) did not agree with this interpretation; he considered crystallisation of the pigments as the cause of the regular forms of the chromoplasts. Meyer believed that the more pigment the chromoplasts contain, relative to the proteinaceous substrate, the more crystalline are the forms. The nearest perfect crystal forms are found, according to Meyer, when the pigment crystals become completely detached from the chromoplasts, as, for example, in carrot cells. Schimper (1883*b*) stated in his reply that Meyer's ideas about the crystallisation of the pigments in chromoplasts were incompatible with the laws of crystallography, and that the crystal-like shape could exist before appearance of the pigments. But later Schimper (1885) had to admit that crystallisation of the pigments does occur in chromoplasts, for their double refraction and pleochroism in polarised light could be explained only in this way. Schimper then distinguished three types of chromoplasts: *a*) those containing protein crystals, the pigments being amorph (in the form of "grana"); *b*) those containing protein crystals as well as pigment crystals; *c*) those containing only pigment crystals and no protein crystals.

Other investigators interpreted the origin of the peculiar forms of chromoplasts in a very different way. Hofmeister (1867) had explained the forms of chromoplasts with drawn out points by different speeds of growth at their pointed ends. Kraus (1872)

and Fritsch (1883) had given still another interpretation, already expressed in a similar manner by Weiss (1866). According to these investigators, the pointed chromoplasts derive from round bodies by a process of vacuolisation and disruption. Vacuoles would displace the pigments of the chromoplasts towards the periphery and, when reaching the surface, disrupt the round bodies. The particular forms of the chromoplasts would then depend upon how the vacuoles fused together and where they came to the surface and disrupted the bodies.

Courchet (1888), like Meyer, regarded crystallisation of the pigments as the cause of the crystalline forms. He cited several plants with pigment crystals, either completely detached from the chromoplasts or still accompanied by a small amount of plastid material. Courchet related the observations made by Fritsch and Kraus to those he had often made himself but interpreted them in a different way. He had found that reddish tables and triangles can fragment into numerous fine needles, sometimes as many as hundreds in one chromoplast. According to Courchet, these crystal needles consist of pure pigment, and their parallel or divergent arrangement determines the shapes of the chromoplasts. The opinion that crystallisation of the pigments is responsible for the forms of chromoplasts was maintained until recently and is expressed, for example, by Guillermond, Mangenot and Plantefol (1933).

IV. RELATIONSHIP BETWEEN CHROMOPLASTS AND CHONDRIOCONTS

Guillermond and others have shown that plastids can develop from mitochondria. Guillermond (1917, 1919, 1933) has also followed the development of chromoplasts from mitochondria in many plants and has described the formation of the carotinoid pigments in sinuous chondrioconts. The origin of the crystalline shapes of chromoplasts was explained by Guillermond (1919, 1933) in the following way. The chondrioconts form little swellings (renflements) in which at first starch grains, then pigment crystals, are formed. The threads combining the swellings are resorbed, and the chromoplasts take on the forms of the pigment crystals.

Guillermond (1919) has discussed also the "protein crystals" (needles) which Schimper (1883*a*) saw in developing chromoplasts and had held responsible for their crystalline shapes.

Guillermond admitted in this connection that the spindle and rod shapes of chromoplasts can not exclusively be explained by crystallisation of the pigments, since leucoplasts often have this form already and also since chromoplasts with spindle and rod shapes are known which contain the pigments in a diffused or granular form. According to Guillermond, these crystal-like structures derive in most cases from chondrioconts (" rectilinear chondrioconts "). Since they do not show birefringence in polarised light, Guillermond doubted that they are crystals. On the other hand, Guillermond was so much impressed by the crystalline shape of some of these plastids, for example, the leucoplasts in the roots of *Phajus grandifolius* or the chloroplasts in the epidermis of the stem of *Cerinthe minor*, that he did not exclude completely Schimper's interpretation and preferred to leave the question in suspense.

Moreau (1915, 1916), who studied the development of chromoplasts in the fruits of *Lycium barbarum* and in the grains of *Taxus baccata*, stated that their shapes (rods, spindles, tadpoles, dumbbells and other roundish forms with drawn out points) are determined by the forms of the chondrioconts from which they develop. Moreau did not mention crystallisation of the pigments as a factor contributing to the form, and has correctly interpreted the determining influence of the chondrioconts on the crystalline shapes of the chromoplasts.

It was mentioned in chapter II that many authors have objected to the theory that plastids derive from mitochondria. It is understandable that conclusions drawn from the observation of small cell granules (chondriosomes) will be controversial. Chondrioconts (rods, filaments, etc.) are more impressive in size and shape than granules are, and it is therefore easier to study their development inside the cells. If chromoplasts really differentiate from chondrioconts (Guillermond, 1917, 1919), study of this development might clarify also the relationship between mitochondria and plastids in general, and might lead to a better understanding of the nature of mitochondria.

V. SHAPES AND DEVELOPMENT OF CARROT CHROMOPLASTS

Most investigators have regarded the carotene bodies of carrots as carotene crystals, either completely detached from the plastids or still accompanied by a small amount of plastid material (Fritsch,

1883; Meyer, 1883*b*; Schimper, 1885; Courchet, 1888). Guillermond, Mangenot and Plantefol (1933) have described the development of carrot chromoplasts from chondrioconts in accordance with Guillermond's (1919) interpretation of the development of other chromoplasts (see the preceding chapter). According to this view, little swellings (renflements) appear at different parts of the chondrioconts. Starch grains form in the swellings, and the threads connecting the swellings are resorbed. A carotene crystal in the form of a needle, a triangular plate or a spiral ribbon is then produced inside the swelling. While the starch grain is resorbed little by little, the plastid takes on the form of the pigment crystal. The mitochondrial wall is resorbed and the pigment crystal set free.

Recently Weier (1942) undertook a detailed cytochemical study of the carotene bodies of carrots. When Weier treated carrot slices with lipid solvents, no residue of the carotene bodies, or only an insignificant amount, remained in most cases. Weier came to the conclusion that the larger bodies were carotene crystals but had some doubt about the smaller ones. Unexpectedly Weier sometimes found that a fibrillar structure recalling that of chloroplasts remained of the larger carotene bodies after cytochemical treatment. In cases where the pigments were associated with starch grains and were not of crystalline appearance, Weier regarded this "cytoplasm-starch-carotene complex" as being of the nature of a plastid.

Roberts and Southwick (1948) have examined carotene bodies of carrots by electron microscopy and have observed the presence of "protoplasmic entities". Vecher (1947) believed to have isolated and analysed chromoplasts of carrots. However, the carotene contents (2.4%) of Vecher's preparations is about one-tenth that of preparations obtained by the present writer, indicating that Vecher's preparations contained an excess of cytoplasmic granules and small fragments of the chromoplasts.

The present writer (Straus, 1939) did not think that the carotene bodies of carrots are crystals of carotene and tried to clarify the question by morphological and chemical analysis (Straus, 1942, 1943). Recently this work could be taken up again, and the structure observed in fragmentating carrot chromoplasts was described on the basis of photomicrographs (Straus, 1950). Very fine fibrils (0.3–0.4 μ diam.) in parallel alignment can sometimes be seen in

disintegrating chromoplasts. The fibrils themselves disintegrate easily because of their almost submicroscopic dimensions. The peripheral portions of the carrot chromoplasts show a more pronounced fibrous organisation ("border fibers") and are therefore better preserved during the disintegration of the bodies. The system of parallel fibrils has a lamellar character. Several lamellae can be superimposed, as recognised by parallel stripes marking the end of layers and by the stronger coloration of superimposed layers. The granular structure is the most difficult to observe, since the "grana" are at the limit of or below microscopic visibility. Where the grana can be seen, they seem to be located at regular intervals from each other and in parallel rows on the fibrils or lamellae.

FORMS OF CHROMOPLASTS AND OF COLORLESS PRECURSORS IN LIVING CARROT CELLS. In the following paragraphs some observations (unpublished until now) by the present writer concerning the forms and development of carrot chromoplasts will be reported. The microscopic observations were made on living cells (cyclosis). Slices from the xylem of older carrots, reddish throughout, were examined in 6–8% sucrose solution until streaming of the cytoplasm ceased.

According to their shapes, carrot chromoplasts can be divided into five groups: *a*) FILAMENTOUS FORMS—Reddish filaments are often found in the xylem cells of carrots. Their length is often striking, the longest ranging from 50–70 μ and extending almost through the whole cell. Some reddish filaments are straight, others are curved in many different ways, for example, in the form of a large 8 or a lasso. Usually, these reddish filaments are about 0.7 μ wide, but occasionally very thin reddish filaments at the limit of microscopic visibility have been observed. The thicker reddish filaments (0.7 μ width) are related to the "border fibers" of crystal-shaped chromoplasts, as will be pointed out below. The "rods" found among isolated chromoplasts are either fragments of these reddish filaments or are "border fibers" split off from crystal-shaped chromoplasts. The color intensity of most of the reddish filaments appears the same as that of crystal-shaped bodies. *b*) ROUND AND CURVED FORMS—Round reddish bodies can occasionally be found in living carrot cells. They are discs, completely or partially pigmented. Much more numerous are reddish filaments closed into circles, "rings", with diameters from 1–10 μ. Very

numerous among this group of curved chromoplasts are reddish sheets and ribbons (to be described below), rolled in, curved or undulated in different ways. *c*) POLYGONAL FORMS—The polygonal forms constitute the great majority of carrot chromoplasts. They are in most cases rectangular (tetragonal) sheets or ribbons of different lengths and widths. On superficial observation and when seen from the side, tetragonal plates may appear as rhomboids, but repeated observation shows that they are rectangular. Besides tetragonal plates, triagonal, pentagonal and hexagonal plates with angles other than 90° also occur. Most of the angles, other than 90°, seem to approach 45° and 135°. The majority of plates and ribbons are flat, but they may also be curved or undulated, as was mentioned above. Reddish filaments (described above) sometimes extend directly into the " border fibers " of the crystal-shaped bodies, thus showing the relationship between the reddish filaments and the crystal-shaped bodies. Often only two parallel " border fibers " delimit the two longer sides of a reddish ribbon, the two other sides remaining " open ". *d*) SPIRAL FORMS —Reddish spiral ribbons and filaments of different sizes are quite numerous among the chromoplasts of carrots. From the point of view of this discussion, this form is the most interesting, since, to the knowledge of this writer, spiral forms occur only in living matter, not in inanimate matter, such as pigment crystals. *e*) MIXED FORMS—Some combinations of straight and round forms have already been mentioned. A few other examples may be reported. Straight reddish filaments may pass into rings or spirals. Flat ribbons may pass into undulated or spiral ribbons. A triangular plate may have two straight sides and one curved (concave). A pentaedric chromoplast with a convexly curved side can be seen in the photomicrograph of a recent article (Straus, 1950, Fig. 12). Tetragonal ribbons may contain fine spirals in their inner part, etc.*

As mentioned above, most investigators have accounted for the forms of carotene bodies in carrots by crystallisation of the pigments. However, neither the filamentous nor the round (curved) nor the spiral forms resemble carotene crystals. Only the small

* An interesting combination of curved and linear forms are chromoplasts with concavely or convexly curved sides and long, drawn out points which occur in other plants but not in carrots.

polygonal forms of carrot chromoplasts may be confused with pigment crystals. Often, however, polygonal ribbons and sheets reach giant size and fill a large portion of the cell, especially in the outer phloem cells of thick carrots. In these cases the forms recall the green chromatophoric sheets and ribbons of certain algae instead of " crystals ".

The results of the morphological examination of isolated chromoplasts (Straus, 1950) suggest that the shapes of chromoplasts are determined by the structure of a protoplasmic framework (stroma). The lamellae, fibrillae and grana observed as part of the stroma are at the limit of or under microscopic size in one dimension at least, while only the " border fibers " are in microscopic range. The fragility of the stroma finds expression also chemically in the low protein content of the chromoplasts. These findings suggest the possibility that carrot chromoplasts derive from colorless precursors of partially submicroscopic size which are easily overlooked during microscopic observations.

It is, however, sometimes possible to recognise colorless precursors of the chromoplasts in living carrot cells (xylem cells): colorless filaments, discs, spirals and polygonal plates. These structures can be identified most easily as precursors of the chromoplasts in those cases where they are partially pigmented, partially still colorless. Very thin and flat colorless discs, for example, may be recognised in which only a segment of the disc or only the peripheral rim contains pigment, and the rest is still colorless. Or a small reddish " crystal " may be found inside a colorless disc, or a ribbon rolled around its periphery; or reddish sheets may have colorless discs lying upon them. The precursors of the large polygonal chromoplasts are more difficult to recognise because of their mostly submicroscopic thickness. Sometimes, however, rectangular portions of the cytoplasm, though not directly visible, appear as rectangular reflections when the objective is focused up and down. It seems that the light reflection of this rectangular portion of the cytoplasm is a little stronger than that of the surrounding part. Peripheral portions of these reflections are sometimes microscopically visible as more strongly organised, colorless rectangular fibers, or as reddish filaments, forming several right angles. Occasionally also an extremely thin rectangular colorless portion can be observed in the " open " end of a reddish ribbon, the ribbon

being delimited in this case only by two parallel " border fibers ". Development of the spiral form is most difficult to follow inside the cells, and no definite conclusions could be reached until now. A few observations on living cells seem to suggest that the spirals take their origin as peripheral portions of the colorless discs, the discs lying in rows behind each other. Other observations, however, seem to indicate the formation of the spiral forms from colorless cytoplasmic areas in spiral movement (see further below).

COMPARISON BETWEEN CARROT AND TOMATO CHROMOPLASTS. At this point a few observations about the development of the rectangular forms of chromoplasts in the fruits of tomato may be included. Though they contain lycopene as their main pigment, the analogies in form and development with the carotene bodies of carrots go very far. After Schimper read the description of the red bodies in tomato cells by Millardet (1876), he (1885) changed his previous opinion, according to which the regular form of these chromoplasts would be caused by " protein crystals ", considered by him as a kind of inactivated protoplasm, and he accepted Millardet's opinion that the colored bodies of tomato cells would be pigment crystals. If the cells of still greenish tomatoes are examined, one comes, however, to the conclusion that Schimper's original interpretation was not too far from the truth, for the rectangular form of developing chromoplasts in tomato cells can often be recognised before the bodies contain pigments. The " stroma " can be distinguished in these cases by the more pronounced organisation of two parallel " border fibers " which may be either colorless or already reddish, while the rectangular inner portion of the chromoplast is not yet directly visible or may be visible but still colorless. It can thus be recognised still easier in tomato cells than in carrot cells that the crystal form of the chromoplasts is independent of the pigments.

DEVELOPMENT OF CARROT CHROMOPLASTS RELATED TO CYTOPLASMIC MOVEMENTS. Examination of xylem cells of carrots with streaming cytoplasm seems to indicate that the colorless fibrils, plates, discs, rings, etc., are originally actively moving cytoplasmic portions which continuously change their forms. The continuously changing forms of the moving cytoplasm in carrot cells duplicate the different shapes of the carotene bodies. The following formative

tendencies, probably related to sol-gel changes, can be distinguished: *a*) round, *b*) linear or angular, *c*) spiral, *d*) round and linear combined. Round forms are represented by systems of "rings" or discs of different diameters, mostly several rings or discs in association, but occasionally also single round masses in amoeboid movement. It is often difficult to see whether the rings are vacuoles or discs of submicroscopic thickness with only the peripheral fiber-like rim in microscopic range. The linear or angular forms are represented by systems of cytoplasmic fibers or polygonal areas ("plates" or "ribbons") delimited by these fibers which continuously change their forms. Here again it can not always be seen whether the fibers enclose cytoplasmic lamellae of submicroscopic thickness, though this is probably the case. Sometimes cytoplasmic fibrils or ribbons in movement show spiral forms. This might be the origin of the spiral shapes of carrot chromoplasts. Mixed forms are found when both round and linear formative tendencies are acting on different portions of the same areas of the moving cytoplasm. It may be emphasised that the round, linear (fibrillar), and polygonal or ribbon-like cytoplasmic areas in movement can also undergo transformations from one form into the other: round forms (discs) into polygonal or ribbon-like lamellae, or inversely straight filaments into "rings" or polygonal plates into discs.

The fibers described above as delimiting the round and polygonal areas of moving cytoplasm are related to or identical with fine plasm streams. Streaming of the fibers may have ceased, though they still continue their movement, forming round or polygonal areas in the cytoplasm. Often several of these fine streams extend in parallel alignment to each other, probably enclosing submicroscopic streaming portions of the cytoplasm between them. If our hypothesis is correct, these ribbon-shaped plasm streams would have caused the forms of the many ribbons of different width found among carrot chromoplasts. They are delimited by only two parallel border fibers on their long sides, the two short sides of the ribbon remaining "open". They can be distinguished from tetragonal chromoplasts of similar forms in which four border fibers surround all four sides of the tetragonal chromoplast. These latter chromoplasts would thus originate from tetragonal areas of the moving cytoplasm completely surrounded by border fibers.

In the splitting chromoplast, seen in the photomicrograph of the recent paper (Straus, 1950, Fig. 9), the movement of two adjacent polygonal areas of the cytoplasm seems to have been arrested during development of the chromoplast. If this interpretation is correct, the form of this chromoplast is determined by the form and structure of gel-like, semi-fluid cytoplasmic areas which solidify during formation of the pigments. This seems to follow more clearly from another photograph of the same chromoplast where the " border fibers " were in focus. It can be clearly seen there that the " border fibers " surrounding the two splitting pieces extend along both sides of the narrow split. If this hypothesis is correct, these are the same fibers which surround the colorless polygonal areas of the moving cytoplasm before the pigments are formed.

It would follow from what has been said that the different shapes of carotene bodies depend upon the variable form (state, structure, movement) of the cytoplasm. The following observation, too, supports this view. It was mentioned above that ring-shaped, filamentous or spiral forms appear less often among carrot chromoplasts than the polygonal, crystal shapes. Observation of carrot cells shows that one of these rare forms (rings, filaments, spirals) is represented often several times among the chromoplasts of one particular cell, while the surrounding cells contain only polygonal chromoplasts. It is suggestive to conclude that the whole cytoplasm of one cell is in a particular state which causes formation of several rings, several filaments or several spirals among its chromoplasts.

When the filaments of carrot cells contain pigments, they usually become rigid and cease their movement. Only very thin fibrils (about 0.3 μ diam.) may be found in oscillating movements when they contain pigments. The non-filamentous chromoplasts are always rigid and mostly remain immobile in the cells. But occasionally, large chromoplasts (plates or ribbons) can be seen moving from one place of the living cell to another. This movement seems to be analogous to the movement of chloroplasts as described by Senn (1908). According to Senn, it is caused by fine cytoplasmic fibrils which are connected with the " peristromium " of the chloroplasts. The in vivo movement of the carrot chromoplasts seems to be caused also by fine fibrils, probably the same as described

above. Senn (1908) stated that carotene bodies of carrots which he considered as carotene crystals would never show in vivo movements as chloroplasts do. This opinion of Senn is not confirmed by the observations of the present writer.

The movements described above are active movements. Sometimes chromoplasts may be seen carried also passively through the streaming cytoplasm. These passive movements can be easily distinguished from active movements and are of no special interest.

VI. RELATIONSHIP BETWEEN CHONDRIOCONTS, CYTOPLASMIC FIBRILS AND PLASM STREAMS

Since examination of developing chromoplasts in living carrot cells points to a close relationship between chondrioconts, cytoplasmic fibrils and plasm streams, some pertinent observations of the literature will be reviewed.

At the beginning of this century, Haberlandt (1901) discussed certain fibrillar structures seen by Nemec in plant cells. Haberlandt found through in vivo examination that Nemec's fibrils were in fact fine plasm streams, similar to those already described by Strassburger and other investigators. The description given by Haberlandt of these fine plasm streams and of their continuously changing forms recalls in many respects those which can be observed in the xylem cells of carrots. Haberlandt mentioned that the streaming may soon cease during observation because of the sensitivity of the object. He thought that the streams function in the transport of material through the cells. Later on, Meves (1917) showed that the fibrils studied by Haberlandt were identical with chondrioconts, since he found them well preserved and stained by the special methods used for mitochondria. Meves thought, however, that these chondrioconts have nothing to do with plasm streams. Meves (1917) also found many other fibers seen by earlier investigators in plant and animal cells, as having the nature of chondrioconts. Recently Scott (1950) has described fine fibrils in plant cells which perforate the membranes of the nuclei and plastids, and function in the traffic of material between nuclei, plastids and cytoplasm. The fibrils observed by Scott are perhaps analogous to those seen by Haberlandt, Nemec and Strassburger.

According to the observations on xylem cells of carrots plasm streams and chondrioconts have to be regarded as closely related,

contrary to the views of Meves. In carrot cells the plasm streams and colorless filaments have the same straight or curved forms, and about the same diameters as the filamentous chromoplasts and the "border fibers" of polygonal chromoplasts. All streaming portions of carrot cells, especially also the more strongly organised outer portions ("border fibers"), are stained vitally by Janus green which is known to stain mitochondria. The "border fibers" of carrot chromoplasts, too, are stained by Janus green.

Recently Buvat (1946, 1947) has observed excessively long chondrioconts (100–150 μ) in tissue cultures of roots of *Cichorium Intybus* in vivo. The chondrioconts described by Buvat seem to have many properties in common with the filamentous chondrioconts of carrot cells (xylem). Buvat mentioned branching, vesiculation, coalescence and formation of alveolar spaces in the long chondrioconts studied by him. They can fragment into short rods and granules, and the granules regenerate into rods. It is interesting that Buvat has also observed a relationship between the forms of the chondrioconts and cyclosis: when cyclosis was weak or had ceased, the chondrioconts were very long; when cyclosis was strong, the chondrioconts had their usual short size. We think that these changes might be related to sol-gel changes in the cytoplasm, the cytoplasm approaching the state of a gel (with long chondrioconts) when cyclosis is weak, and approaching the state of a sol (with short chondrioconts) when cyclosis is strong. Though Buvat observed an apparent disappearance of mitochondrial substance into the cytoplasm during fragmentation of the long chondrioconts, he believed that the mitochondria always preserve their individuality and are not formed de novo. The chondrioconts observed by Buvat and the present writer in plant cells recall similar forms of chondrioconts described by M. R. Lewis and W. H. Lewis (1915) in the cells of tissue cultures of chicken-embryos.

The colorless area in movement (filaments, discs, ribbons, polygonal area, etc.), appearing in xylem cells of carrots and regarded as precursors of chromoplasts, can be considered either as mitochondrial structures similar to those seen by Buvat (1946, 1947) in plant cells and by Lewis and Lewis (1915) in animal cells, or as special forms of (lamellar) leucoplasts. It may be pointed out in this connection that lamellar forms of chloroplasts (thin discs) have occasionally been reported in the literature (Rotherth, 1912;

Senjaninova, 1926). The existence of polygonal chloroplasts (v. Mohl, 1855; Sachs, 1862; Hofmeister, 1867; Stahl, 1880) would also support this interpretation. It would be also possible to regard the colorless areas of the xylem cells of carrots as portions of the moving cytoplasm in a particular functional state, associated with sol-gel changes. They would become rigid (fixed) during the synthesis and accumulation of the pigments.

It is now possible to understand much better the observations made by Weiss (1864), Kraus (1872), Fritsch (1883), Schimper (1883) and Guillermond (1919) during the development of crystal-shaped chromoplasts. Kraus (1872) and Fritsch (1883) explained the origin of the crystalline shape of chromoplasts through the formation, fusion and disruption of vacuoles inside roundish plastids. Since Kraus and Fritsch have mostly studied chromoplasts with rounded sides and drawn out points, the phenomena observed by them would correspond to those mentioned above as the movement of round forms (vacuoles or discs). The curved sides of these pointed chromoplasts would thus have been determined by systems of "rings" (vacuoles or discs) of different diameters to be found in the stroma of the chromoplasts or in the cytoplasm adjacent to the chromoplasts. Schimper (1883), on the other hand, had related the regular forms of the chromoplasts to "protein-crystals" (spindles, needles) which he considered as the protoplasm of the plastids in an inactive state. Schimper's protein crystals may be compared with analogous structures described by Weiss (1864) in spindle-shaped chromoplasts. Weiss spoke of "schleimige farblose Fortsätze, die Reste ursprünglicher Plasmaströme". In comparing the drawings made by Schimper and Weiss, it becomes clear that both saw the same structures: the "protein crystals" (needles) of Schimper are identical with the "rests of plasm streams" of Weiss. Guillermond (1919) recognised that the "protein needles" of Schimper are "rectilinear chondrioconts" (see chapter IV). When these same chondrioconts have been transformed into chromoplasts, however, Guillermond does not recognise them any more as such but speaks of pigment crystals (needles) consisting of pure pigment. The chromoplasts do not take on the form of pigment crystals, as Guillermond says, but inversely, the pigments crystallise in the form of the chondrioconts or related cytoplasmic structures (discs, rings, plates, spirals, etc.) This poses a problem as to the state

of the pigments at different stages of chromoplast development which will be discussed below.

Lewitsky (1926) and Senjaninova (1927) have described so-called mitochondrial bodies in the germ cells of lower plants. These bodies may be understood as the stromata of chromatophores, containing no or only little pigment. Senjaninova (1927) has given an interesting description of the mitochondrial body in the archesporium cell of a moss (*Catharinea undulata*). This body has a lamellar and fibrillar structure and shows at certain stages "mitochondria" (grana?) arranged in parallel rows and connected through finest filaments. During cellular division the body divides into two parts, one of which is distributed to each daughter cell. In the young spore the mitochondrial body is found transformed into chloroplasts from which new chloroplasts derive by division. They still show the fibrillar and lamellar, but no more the granular, structure. The "mitochondrial body" described by Senjaninova recalls the stroma or the precursors of carrot chromoplasts.

VII. COMPARISON OF THE SUBMICROSCOPIC STRUCTURE OF CHROMOPLASTS AND CYTOPLASM

The above described observations on carrot cells seem to indicate a close relationship between cytoplasmic fibrils and chondrioconts, and between the structure of the cytoplasm and of chromoplasts. It has been postulated by several investigators, especially by Wrinch (1941), that fibrous crystalline associations of protein molecules (gels) are part of the basic structure of protoplasm. Recently the significance of the fibrillisation of protein molecules (rod-shaped or globular) for the structure of protoplasm has been discussed by Kopac (1951). Submicroscopic fibrillar structures have actually been observed in the cytoplasm of several animal cells by electron microscopy (Porter and Thompson, 1947; Hillier, Mudd and Smith, 1949; Bang and Gey, 1948; Pallade, 1952*a*) and in plant cells by Bretschneider (1950). It may also be mentioned in this connection that Needham (1942) compared the structure of protoplasm to a liquid crystal and that he related this conception to the morphogenetic development of organisms.

In carrot chromoplasts the submicroscopic, crystalline and fibrillar structure of the cytoplasm seems to be partially preserved under the protective layer of the pigment. According to Monné (1948), the cytoplasmic fibrils contain at regular intervals little nodules,

"chromidia", in which the protein chains are folded and ribonucleic acid is concentrated. This disposition of the chromidia seems to correspond to the arrangement of (submicroscopic) grana on the fibrils of carrot chromoplasts. The structure of the resting nucleus as seen by Strugger (1947) with the phase contrast microscope also recalls the structure of the cytoplasm and chromoplasts. Strugger found the chromomeres of the resting nucleus in parallel rows resembling the lattice of a crystal. The grana of the plastids seems to be arranged in a similar way. The fibrillar and helicoid structures would be other points of analogy between chromosomes and the fibrils of cytoplasm and of chromoplasts. Submicroscopic fibrils have been observed also in the stroma of spinach chloroplast by Thomas, Bustraan and Paris (1952) by electron microscopy, and helicoid fibrils connecting the grana in several other chloroplasts by de Rezende-Pinto (1948, 1952). The fibrils of the chloroplasts were compared by Thomas, Bustraan and Paris (1952) and by Bustraan, Goedheer and Thomas (1952) with cytoplasmic fibrils, and by de Rezende-Pinto (1948) with the chromonemata of the nucleus ("chloroplastonema"). According to this hypothesis, there would exist a close analogy in the basic structure of the nucleus, cytoplasm and plastids. The cytoplasm of carrot cells and the stroma of carrot chromoplasts are rich in ribonucleic acid. Perhaps this substance has an influence on the shapes of the chromoplasts in analogy with the nucleoproteins of tobacco mosaic virus which also form rods, crystals and "tactoids" (Bernal and Fankuchen, 1941).

The existence of two-dimensional order (lamellae) seems to be of special importance in plastids (Frey-Wyssling, 1948). The lamellar texture of chloroplasts has been seen by Menke (1940*a*) in the ultraviolet light microscope and by Strugger (1947) in the phase contrast microscope. As mentioned above, very thin discs were found also in association with developing chromoplasts in carrot cells. The carrot chromoplasts themselves sometimes reveal their lamellar structure through stripes indicating the ends of the layers; rows of tiny discs were observed in the stroma (Straus, 1950, Fig. 11). Submicroscopic discs (10 mμ thickness, 50 mμ–5 μ diameter) have been found by Menke (1940*b*) and Kausche and Ruska (1940) in chloroplasts by electron-microscopy. These authors considered the discs as part of the fundamental structure of the chloroplasts. Algera et al. (1947) and Frey-Wyssling and

Mühlethaler (1949), however, regarded these discs as myelin forms from disintegrating chloroplasts. Recently Steinmann (1952) has been able to decide this question by demonstrating in electron-micrographs submicroscopic lamellae in the grana-free chloroplasts of *Spirogyra* as well as in the grana of the chloroplasts of *Aspidistra*. Perhaps the lamellar structure of the plastids is related to surface reactions of enzmatic nature which take place in the plastids.

It would follow from what has been said that the crystalline forms and structure of chromoplasts are determined primarily by gel-like crystalline associations of protein molecules, similar to those which seem to form also the basic structure of the protoplasm. On the other hand, cytoplasm contains also considerable amounts of lipids, as is known, for example, from analysis of isolated cytoplasmic fractions (mitochondria and microsomes). In carrot chromoplasts the amount of lipids even exceeds the amount of proteins. It is difficult to evaluate the influence of the lipids on the structure of the chromoplasts as long as their degree of orientation is not known. As was discussed earlier (Straus, 1942), the structure of carrot chromoplasts may be similar to that of the myelin sheets of nerve fibers and of the external segments of the rods of the retina, the latter containing also a carotinoid pigment, bound to protein, the visual purple. According to W. J. Schmidt (1937), these structures are composed of alternating layers of proteins and perpendicular lipid molecules. Wald (1950) has observed longitudinal and cross striations in isolated rod outer segments from the retina of frogs, and Luna (1913) has described the derivation of the retina pigments in *Bufo* from chondrioconts. Sjörstrand (1949) has demonstrated the submicroscopic lamellae of the rod outer segments of the guinea-pig retina by electron-microscopy after sonic treatment. A similar lamellar structure has been found by Sjörstrand (1950) and Fernández-Morán (1952) in the myelin sheets of the nerve fibers by electron-microscopy. In chloroplasts, too, alternating layers of proteins and lipids have been postulated (Frey-Wyssling, 1948).

VIII. HYPOTHESIS ON THE DEVELOPMENT OF MITOCHONDRIA AND CHROMOPLASTS FROM SUBMICROSCOPIC PRECURSORS

The foregoing speculations about the submicroscopic structure of carrot chromoplasts and the observations on the development of

chromoplasts in carrot cells seem to support the early views of botanists that plastids develop directly from the cytoplasm. Fibrils, discs, plates, etc., which probably contain mitochondrial material and are precursors of chromoplasts, seem to arise de novo in the cytoplasm of carrot cells. This seems to contradict the now generally accepted opinion that mitochondria and plastids derive only from preexisting mitochondria and plastids. Both views may be combined if one considers the possibility that mitochondria and plastids can develop from microscopic as well as from submicroscopic precursors. Thus plastids seem to differentiate from the cytoplasm when the precursors have submicroscopic size; they appear to develop from mitochondria or leucoplasts when the precursors have microscopic size.

This view seems to be corroborated by recent electron-microscopic studies on animal cells, indicating that mitochondria themselves may have submicroscopic size or contain submicroscopic constituents of the size of the "microsomes" (Claude and Fullam, 1945); Dalton et al., 1949; Pease and Baker, 1950). The possibility of a genetic relationship between submicroscopic particles and mitochondria was discussed also by Claude (1944) who observed that submicroscopic particles could be recovered from disintegrating mitochondria. Similar observations were made recently by Harman (1950) who thought that the structure of mitochondrial rods might be that of a tightly coiled or compact gel. Grave (1937) has studied the ultrastructure of the chondrioconts in renal tubule cells of amphibia in polarised light. He concluded that the batonettes are composed of protein particles or fibrillae extending parallel to the axis of the chondrioconts, and of lipid molecules oriented perpendicular to the axis. Pallade (1952*b*) made important electron-microscopic observations upon the submicroscopic structure of mitochondria from various animal cells which show that mitochondria contain a system of submicroscopic parallel lamellae, more or less perpendicular to the long axis of the mitochondria.

The development of mitochondria from submicroscopic precursors is also suggested from examinations of living cells. As reported in chapter VI, the observations of Buvat (1946, 1947) on plant cells and of M. R. Lewis and W. H. Lewis (1915) on animal cells have shown an apparent disappearance of mitochondria

into the cytoplasm in vivo. Recently the observations of Lewis and Lewis were confirmed by Frederic and Chevremont (1952) by following the movements of chondrioconts in the cells of tissue cultures of chick embryos cinematographically. The films revealed the great plasticity of the chondrioconts in vivo, for example, development of filaments from granules, exchange of mitochondrial material with the cytoplasm and the nucleus, and complete disappearance of some mitochondria into the cytoplasm. The important experiments of Harvey (1946) also showed the close relationship. It was found by Harvey that the " clear quarter " of the fertilized *Arbacia punctulata* egg which did not contain large granules developed normally and that the mitochondria seemed to arise de novo from the ground substance in the pluteus. Gustafson and Lenique (1952) also observed the formation of mitochondria from small precursors in developing sea urchin eggs. In plant cells (myxomycetes and mosses) the decrease of the size of mitochondria towards or beyond microscopic visibility was described by Lewitsky (1924) and Senjaninova (1927*b*).

It has been assumed that mitochondria possess a certain degree of autonomy (Meves, 1908; Woods and duBuy, 1951). According to Wilson (1925), mitochondria must have the property of unlimited growth by self-duplication. Since microscopically visible mitochondria do not in most cases show any regular forms of division, the process of self-duplication may take place in submicroscopic constituents or precursors of the mitochondria, perhaps related to the " microsomes ". Payne (1952) observed within mitochondria of adrenal cells of fowl, small granules which seemed to divide. The possible relationship between microsomes and mitochondria was also discussed recently by Newcomer (1951). The opinion that microsomes may have the character of self-duplicating units and be related to viruses is expressed by Brachet (1947) and Monné (1948). Lehman (1947) considered cytoplasmic fibrils, mitochondria and chromonemata as the fundamental vital elements of the cell, " biosomes ", and related to viruses.

Plastids are known to have independent properties, since mutation of plastids can be inherited independently of the nucleus (Imai, 1928; Rhoades, 1943). If the submicroscopic fibrils of chromoplasts were analogous to chromosomes, and the grana analogous to chromomeres, the genetic properties of plastids could be better

understood. By discussing possible analogies in the structure of plastids, cytoplasm and nucleus, the predominant influence of the nucleus on heredity will, of course, not be contested.

A relationship between plastids and viruses has been postulated by duBuy and Woods (1943, 1951). According to these authors, virus infection causes characteristic changes in plastids, and variegations of plastids resemble virus infections. Kausche and Ruska (1940) have found tobacco mosaic virus localised in chloroplasts and concluded that the virus protein and plastid protein are related. According to the observations of Black, Morgan and Wyckoff (1950), chloroplasts of leaves infected with tobacco mosaic virus contain fibrous masses formed by end to end arrangement of the virus rods. In an earlier paper the present writer indicated morphological similarities between carrot chromoplasts and virus inclusion bodies (vacuolisation, striation, fragmentation) (Straus, 1942). Another striking analogy between virus inclusion bodies and carrot chromoplasts can be seen in the lamellar and helicoid structure as revealed for the inclusion bodies of tobacco mosaic virus by Wilkins, Stokes, Seeds and Oster (1950), and discussed for carrot chromoplasts in the present paper. As will be reported elsewhere, the protein fraction of isolated carrot chromoplasts was found to be rich in ribonucleic acid. This property further approaches the stroma or the precursors of chromoplasts to viruses.

It is easier to find morphological analogies between different constituents of the cell than to understand their significance for metabolism, growth and differentiation. Little is known about the metabolic processes accompanying the development of chromoplasts and about the formation and role of the pigments. The possible relationship between intra-cellular fibrillisation and gelation to energy-coupling systems involving ATP was pointed out by Kopac (1951). We think that similar enzymatic processes may be connected with the streaming and fibrillisation of cytoplasm and the development of chromoplasts observed in the xylem cells of carrots. Much more has to be learned about this before structure can be related to function.

IX. STATE OF THE PIGMENTS IN CHROMOPLASTS AND MICROSCOPIC STRUCTURE OF PLASTIDS

MICROSCOPIC OBSERVATIONS CONCERNING THE STATE OF THE PIGMENTS IN CHROMOPLASTS. Schimper (1885) studied the

chromoplasts of a great number of plants and made many observations relating to the microscopic aspect of the pigments. Courchet (1888), who examined the chromoplasts of numerous other plants, confirmed Schimper's observations. The following description is based upon their data.

The pigments of chromoplasts can be found either in the amorphous or in the crystalline state. When the pigments are amorphous, they are contained in so-called " grana ", viscous droplets filling out tiny vacuoles in a colorless " stroma " (Schimper, 1885). The grana appear in shades of yellow, brown, orange and red. The number of the grana is very variable; a chromoplast may contain a few large or many small grana, or intermediate amounts. The pigments can appear microscopically also in homogeneous distribution in chromoplasts with yellow, orange or red colors (Courchet, 1888). In these chromoplasts the grana are perhaps too small or too densely packed to be seen. The same chromoplast may contain part of the pigments in homogeneous distribution, another part in more deeply colored granules.

The crystallised state of the pigments in chromoplasts can be recognised, according to Schimper, by the pleochroism and double refraction in polarised light. The pigment crystals are usually much larger than the grana but sometimes do not exceed their size. The same chromoplast may contain grana and crystals of the same color, or yellow grana and small red crystals. The pigment often appears at first in the form of grana, later in crystals; but sometimes it is already crystallised as soon as it can be seen. The pigment crystals often are bundles of very thin needles which may be straight or curved and interwoven in many ways, depending on the different many-pointed forms of the chromoplasts (Schimper, Courchet). According to Courchet, pigment crystals can become completely detached from the plastids. In the cells of the fruits of *Cucurbita Pepo* (red variety), for example, reddish ribbons are formed in the periphery of leucoplasts, then detached. Plates originate by transversal fragmentation of the ribbons. Courchet thought that these bodies consisted of pure pigment, and he considered the state of the pigment as intermediate between the crystalline and amorphous states in view of the sinuous forms. However, the pigment bodies in the cells of *Cucurbita* have the same shapes (plates, ribbons, spirals, rods) as the chromoplasts of carrots, and probably have also a similar or identical composition.

Courchet noted that the pigments sometimes are concentrated in the periphery of the plastids, either as rows of granules or as homogeneous rings, and that these peripheral portions of the plastids sometimes separate and give rise to rods and spindles.

Courchet tried to relate color and form of the chromoplasts but no general rule seemed to exist. Though yellow colors occurred usually in roundish chromoplasts, less often in rods, Courchet also found roundish chromoplasts with orange or red colors, the pigments either appearing in granules or in homogeneous distribution. The three- and many-pointed chromoplasts were found by Courchet mostly in orange colors.

Guillermond (1917), too, has studied the formation of the pigments in chromoplasts and chondrioconts microscopically, and has cited the following types and examples: *a*) The pigment is produced in a diffused state or in the form of fine granules in mitochondria (chondrioconts) or in chromoplasts developed from mitochondria. Examples: In the cells of the petals of *Tulipa suaveolens*, chondrioconts (long and flexible filaments) contain the yellow pigments in a diffused state. In the cells of the fruits of *Asparagus officinalis*, roundish chromoplasts, formed by vesiculation of chondrioconts, contain many red granules. *b*) The pigment is originally found in a diffused or granular state in mitochondria, or chromoplasts developed from mitochondria, and later on crystallises. Examples: In the cells of the petals of *Gladiolus* the chondrioconts are impregnated with a pale yellow pigment in a diffused state. Later the color turns into yellow orange, and part of the pigment crystallises in the form of a long needle extending all along the chondriocont. In the petals of *Clivia nobilis* the red orange pigment appears in elongated chondrioconts at first as fine granules, later crystallised in the form of a needle. *c*) The pigments are found in diffused, granular or crystalline states in large chromoplasts, developed by metamorphosis of chloroplasts which on their part have derived from mitochondria. This case is realised in the mesocarp and mesophyll of most fruits and flowers.

According to Schimper (1885), the structure of chromoplasts and chloroplasts is analogous; both contain the grana with the pigments embedded in a colorless stroma. Schimper (1885) and Courchet (1888) have observed transition forms between chloroplasts and chromoplasts which reveal the state of the green and

red pigments in the same plastids. In examining such plastids in the cells of *Cucurbita Pepo* (yellow variety), Courchet (1888) made the interesting observation that these cells contain elongated plastids with " rods of chlorophyll " and an orange colored ring around the periphery. Rotherth (1912) studied the chromoplasts in the vegetative organs of about 200 tropical plants and found among them all stages of transition between chloroplasts and chromoplasts, the shapes of the plastids being mostly roundish. While the carotinoid pigments were usually localised in distinct grana, the chlorophyll seemed to impregnate the stroma of the same plastids in homogeneous distribution. Rotherth thought that the grana structure was valid only for chromoplasts, or that the grana of chloroplasts must have submicroscopic size. It may be recalled, however, from the observations of Schimper, Courchet and Guillermond that the carotinoids, too, can appear microscopically in homogeneous distribution in plastids.

Savelli (1937*a*), also, has found many transition forms between chromoplasts and chloroplasts in tropical plants. While the chlorophyll mostly appeared in homogeneous distribution, the carotinoid pigments presented various aspects. It may be of interest to cite the following examples, observed by Savelli. In the leaves of *Gasteria nitida* (very green) chloroplasts contained five to ten large red droplets in which the carotinoids seemed to be dissolved. Some chloroplasts with similar but colorless droplets were stainable with Sudan, and Savelli thought that the carotinoids had been taken up by these lipid droplets after their having been originally localised in the stroma in homogeneous distribution (" chlorolyse intraplastidienne "). In the leaves of *Kniphofia* the plastids showed twelve to 30 or more reddish-brown granules which appeared dense, not liquid as in *Gasteria*. The color seemed to indicate that each granule contained both chlorophyll and carotinoid pigments. In the leaves of *Aloe*, Savelli observed in (often deep green) chloroplasts reddish granules, rods or spindles, the red pigments thus being in solid form and separated from the chlorophyll. In other chromoplasts (*Aloe latifolia, Gasteria maculata, Haworthia*) Savelli was sometimes able to distinguish the carotinoid pigments as extremely fine points at the limit of microscopic visibility. After having observed such different appearances of the carotinoid pigments, Savelli poses the following questions: What

really are the grana? Carotinoids in the solid state (crystalline or amorphous)? Pigments dissolved in lipid droplets or bound to proteins? Carotinoids alone or associated with the other chloroplast pigments? Embedded in the stroma or part of the stroma? Each possibility seemed to find support in Savelli's above cited observations.

Weber (1937) examined forms intermediate between chloroplasts and chromoplasts in polarised light. The leaves of *Adoxa Moschatellina* contained plastids with green and red grana. While the green grana were difficult to visualise, the red grana were distinct. In polarised light the green portion of the plastid radiated green light while the red grana remained dark. After treatment with sodium oleate, the green part of the plastid formed myelin figures, the red grana remained unchanged and seemed somehow to be held together. Similar observations were made by Weber on chromoplasts of *Selaginella*.

In developing carrot chromoplasts, examined by the present writer, the beginning of pigmentation can sometimes be seen as pale yellowish color diffused all over the stroma. In other chromoplasts of carrots, however, the pigment appears in the beginning in a few tiny red granules at the limit of microscopic visibility. As may be concluded from the irregular movements after their detachment from the stroma, the shape of these granules must be crystalline. The pigment seems already crystallised in these granules as soon as it can be seen (reddish color). In fully developed carrot chromoplasts, the pigments appear in homogeneous distribution with orange or red colors. It is difficult to conclude from microscopic observations alone whether the homogeneous aspect of the pigments is caused by many submicroscopic or densely packed granules, or by a single crystal covering the stroma.

PHYSICAL STATE OF THE CAROTINOIDS IN CHROMOPLASTS. No systematic studies have been made about the physical state of the carotinoid pigments in chromoplasts. The microscopic aspect of the pigments, described by Schimper (1885), Courchet (1888), Guillermond (1917) and Savelli (1937*a*), and reviewed in the preceding chapter, has shown that the physical state (degree of dispersion) of the pigments in chromoplasts varies greatly. The state is probably influenced by association of the pigments with lipids or proteins. Certain conclusions can be drawn from the

absorption spectra (colors), from analogies with colloidal solutions and from properties of extractability with organic solvents.

The absorption spectra (colors) of the pigments before and after their extraction from the chromoplasts can give an indication whether the pigments are dissolved in lipids or are present in a colloidal state of different degrees of dispersion. When, for example, the chromoplasts have orange or reddish colors, and when the pigments are extracted from these chromoplasts with yellow color by organic solvents, the orange or reddish colors of the plastids must have been caused by the low degree of dispersion of the pigments. Carotene in chromoplasts of carrots and probably the carotinoids of many other chromoplasts present examples of this kind. When, on the other hand, the pigments are dissolved in lipids inside the chromoplasts, the absorption spectra will be found similar for suspensions of isolated chromoplasts and for extracts of isolated chromoplasts by organic solvents. No studies to this effect were found in the literature, and it can therefore not be indicated whether the dissolution of carotinoids in lipids occurs often in chromoplasts, and in particular whether the " amorphous " state of the pigments in the grana (Schimper, 1885) corresponds to a dissolution in lipids. As was indicated in the preceding chapter, Savelli (1937*a*) found the red pigments of the chromoplasts of *Gasteria nitida* in lipid droplets. Savelli thought, however, that the pigments had originally been dispersed in the stroma and that they were only secondarily taken up by lipid droplets. Perhaps dissolution of the carotinoids in lipids occurs rather seldom in chromoplasts in vivo. After disintegration of the cells and chromoplasts by boiling or maceration, however, the carotinoids can be taken up by lipids, as already indicated by color changes from reddish to yellowish.

When the carotinoid pigments are present inside the plastids in a colloidal state of different degrees of dispersion, they may be adsorbed to proteins. This state may be similar to that in which carotene is adsorbed to Al_2O_3, filter paper pulp or serum albumin. After evaporating, for example, an ether solution of carotene on these materials on a watch glass, the carotene remains adsorbed with reddish color. Still nearer to the natural state is probably the state in colloidal solutions of different degrees of dispersion. It may be worthwhile to review shortly the properties of colloidal

carotene solutions in relation to the degree of dispersion and accompanying proteins and lipids (Karrer and Straus, 1938; Straus, 1939).

Colloidal carotene solutions of high degree of dispersion (small pigment particles) are clear and yellow (absorption maxima around 510 mμ and 475 mμ). With decreasing degree of dispersion (increasing size of the pigment particles), the colloidal solutions become increasingly cloudy and the color changes from yellow over orange to red (absorption maxima around 535 mμ and 486 mμ). In the orange and red solutions, the pigment particles are present as tiny crystals at the limit of microscopic visibility, in the yellow solutions as submicroscopic crystals partly visible at dark field observation. Colloidal carotene solutions of different degrees of dispersion are prepared by varying the concentration of the pigment during the preparation. When the concentration of the pigment is low, the degree of dispersion remains high; with increasing concentration, the tendency of the pigment to form larger particles becomes greater. Accompanying proteins and lipids have marked effects upon the physical state of the pigment in colloidal solutions. In the presence of lecithin, for example, completely clear aqueous solutions can be obtained in which the absorption spectrum of the carotene (maxima at 487 mμ and 456 mμ) is similar to that of carotene in organic solvents. When prepared in a different way, colloidal carotene solutions containing lecithin may show the pigment particles at a lower degree of dispersion (orange solutions), while the lecithin exercises a stabilising effect on the pigment particles. Proteins also stabilise the colloidal carotene particles. While the pigment particles would flocculate in aqueous suspension at higher concentration, the stabilising effect of proteins keeps the tiny pigment crystals in suspension and allows the existence of low dispersed (red) pigment suspensions. In the presence of proteins, the pigment is flocculated by $(NH_4)_2SO_4$ together with the proteins, and the dried precipitate is soluble in water. The pigment can not be extracted with ether from the solution. When lecithin is added, the lecithin is precipitated by $(NH_4)_2SO_4$ together with the proteins and the carotene. The pigment can not be extracted with ether from the dried precipitate when the proteins exceed the lecithin in the precipitate. The pigment becomes gradually more extractable from the dried precipitate, the more the lecithin exceeds the proteins.

It is suggestive to think that during the development of chromoplasts similar changes in the degree of dispersion of the pigments take place as described above for colloidal carotene solutions. As long as the concentration of the pigments is low in the plastid, the degree of dispersion is high. When more pigment is synthesised by the chromoplast, the highly dipersed pigment particles become larger, and crystals ranging from colloidal to the beginning of microscopic dimensions are formed. When the concentration of the pigments increases still more, crystals of microscopic size are formed inside the plastids. This process may be aided by the decrease of proteins concomitant with the accumulation of the pigments and diminishing the stabilising influence of the proteins on the colloidal pigment particles.

This hypothesis would make it understandable that pigments crystallise in the forms of the chromoplasts (stroma) as discussed earlier in this review, and that the reverse process does not take place according to which the chromoplasts take on the forms of the pigment crystals (Meyer, 1883*a*; Guillermond, Mangenot and Plantefol, 1933). It has to be pointed out that the gradual decrease in the degree of dispersion of the pigments could take place as well in small areas of the plastids of the size of the grana as over larger areas such as fibrils or plates.

Savelli (1937*b*, 1937*c*) has studied the double refraction and pleochroism in polarised light of chromoplasts of *Dracaena Draco* (ripe fruits) which have the forms of spindles, triangles, etc. Savelli's conclusions concerning the state of the pigments were similar to those outlined above: the pigments might be present in submicroscopic crystals arranged in the stroma in an orderly fashion. Savelli, too, considered the possibility that the degree of dispersion of the pigments decreased gradually, beginning from submicroscopic dimensions to microscopic dimensions and finally to long parallel needles. Savelli also criticised the opinion according to which the crystal forms of chromoplasts are caused by crystallisation of the pigments. He pointed out that all molecules (proteins, lipids, pigments) contribute to the structure, and supported this by the fact that an almost fibrillar structure could sometimes be distinguished in the chromoplasts of *Dracaena*.

Fully developed carrot chromoplasts appear in two colors, orange and red, as Schimper noticed as early as 1885. These colors also may be due to differences in the degree of dispersion, perhaps

connected with variations in the amounts of accompanying proteins and lipids, or with variations in the amounts of the pigments themselves.

Until now only the properties of colloidal carotene have been well known and could be compared with the state of the carotene in chromoplasts. The colloidal properties of other carotinoids may be quite different from those of carotene, and this may partly explain differences in the physical states of the various carotinoid pigments in chromoplasts. Colloidal xanthophyll solutions, examined briefly (Straus, 1939), could not be obtained in a low dispersed reddish form but only in yellow solutions. According to Guillermond (1917, 1919), xanthophyll occurs also in chromoplasts only in yellow colors. Comparison of the state of other carotinoid pigments in chromoplasts and in colloidal solutions would probably permit interesting conclusions.

Secondary changes in the degree of dispersion of the pigments in chromoplasts can be induced through various reagents and by heating. Such changes were observed by the writer when carrot slices or sediments, containing chromoplasts and cytoplasmic granules, were treated with 70–90% acetone solutions (Straus, 1943), or when purified chromoplast suspensions were heated for a short time without acetone. Microscopic observation showed that in these cases a part of the chromoplasts had faded, and that their pigment had been deposited on other chromoplasts which thereby became more intensely colored, or that the pigment was deposited on granular debris of stromata which became stained reddish. The pigment accumulated at these places can form crystals often several times larger than the original chromoplasts. In a similar way van Wisselingh (1915) induced crystallisation of the carotinoid pigments of several chromoplasts inside the cells after treatment with diluted alcohol or after heating. The reactions employed for histochemical characterisation (crystallisation) of the carotinoid pigments in plant tissues with alcoholic NaOH (Molisch, 1896), resorcin (Tswett, 1911) and phenol (v. Wisselingh, 1915) are probably based on similar alterations in the physical state of the pigments. Meyer had observed the contraction and the formation of pigment crystals (spindles) from the chromoplasts of *Eccremocarpus* (Meyer, 1883a) and of *Tropaeolum Lobbianum* (Meyer, 1883*b*) after treatment of the tissue with glacial acetic acid or with

glacial acetic acid + chloroform (2:1). Since the forms of these pigment crystals were similar or identical to the original forms of the chromoplasts, Meyer concluded that the crystallisation of the pigment must have determined the crystalline shapes of the chromoplasts. This interpretation is probably erroneous. As was indicated above, the pigment dissolved from some chromoplasts by the solvents can recrystallise on others without altering the shapes of these chromoplasts. Menke (1940*c*) has observed a deep red residue of carotene remaining of chloroplasts after extraction of the other pigments by methanol, and he has discussed the possible identity of this state of carotene with the natural state. It is probable that also in Menke's experiment secondary changes in the state (degree of dispersion) and finally crystallisation of the carotene had occurred through the action of the methanol. All these changes in the physical state of the carotinoid pigments in chromoplasts through experimental treatments can best be understood by assuming that the reagents or the heat break the linkage (of adsorption) between the pigments and constituents of the stroma (probably the proteins), and that gradual decrease of the degree of dispersion, and finally formation of large crystals ensues.

Certain indications about the state of the pigments can be obtained also from properties of extractability with organic solvents. As in colloidal carotene solutions, the extractability of carotinoid pigments from chromoplasts is influenced by accompanying proteins and lipids, and also by the degree of dispersion of the pigments. Kuhn and Bielig (1940) and Kreula (1945) have made conclusions on the linkage of the carotene to proteins in carrots by examining the extractability of the pigment from a carrot juice by organic solvents. These authors did not consider, however, that the proteins which prevent extraction of the pigments from carrot juice can be inside or outside the chromoplasts. Conclusions on the natural state of the pigments from properties of extractability can be made only by using suspensions of purified chromoplasts, not with the whole plant extract. The difficult extractability of the carotene from carrot juice by ether, for example, is due mainly to cytoplasmic granules adsorbed to the chromoplasts. Suspensions of purified chromoplasts yield their pigments more easily to ether than does the whole carrot juice. A small amount of carotene, however, can not be extracted by ether from a suspension of

isolated carrot chromoplasts, this portion of the pigments probably being bound to the proteins of the stroma. Since the carotene is present in these chromoplasts in a crystalline state (low degree of dispersion), it is extracted more easily than if it were present in a more highly dispersed state. In carrot cells and in carrot juice, a small proportion of the carotene is contained in cytoplasmic granules. In these granules the carotene is in a more highly dispersed state (yellowish color) and can be extracted by ether only with difficulty.

MICROSCOPIC STRUCTURE OF PLASTIDS. Since chloroplasts and chromoplasts have the same structure (Schimper, 1885), and since the grana have been studied more intensively in chloroplasts, some observations of the literature concerning the microscopic structure and the grana of chloroplasts may be cited in this chapter. For a more detailed discussion of the structure of chloroplasts, see the excellent recent review by Weier and Stocking (1952).

The grana structure of chloroplasts was described by Meyer (1883*b*) and Schimper (1885), and rediscovered by Doutreligne (1935), Weier (1936), Heitz (1936) and Geitler (1937). Most investigators have believed that the pigments are localised exclusively in the grana and that the stroma is colorless. According to Heitz (1936), the grana have discoid shape, and their size varies from 0.3–2 μ; the large grana, especially found in shadow plants, would originate from association of small grana and vice versa. Heitz (1936) and Geitler (1937) considered it possible that the homogeneous aspect of many chloroplasts might be due to the submicroscopic size of the grana. Woods and duBuy (1951) have observed very large grana in mutant chloroplasts and in plastids of virus infected cells. According to electron-micrographic studies by Frey-Wyssling and Mühlethaler (1949), by Thomas, Bustraan and Paris (1952) and by Steinmann (1952), the grana of chloroplasts consist of many superimposed lamellae (discs).

Not much is known about the stroma in which the grana of chloroplasts are embedded. Weier and Stocking (1952) suppose that the stroma may be greatly modified by dehydration and fixation. Electronmicrographs by Granick and Porter (1947) and Frey-Wyssling and Mühlethaler (1949) show the grana very clearly but only disintegrated portions of the stroma. According to Frey-Wyssling and Mühlethaler (1949), the stroma would

contain macromolecules of 200–300 Å diameter in the sol state, but the possibility of a labile gel structure in the stroma would not be excluded. While Frey-Wyssling has limited the lamellar structure to the grana, Menke (1940*a*) has attributed the lamellar structure to the stroma and has considered the grana as an expression of the inhomogeneous distribution of constituents of these lamellae. This question has been clarified by Steinmann (1952) who demonstrated the lamellar structure of the grana-free chloroplast of *Spirogyra* by electron-microscopy.

The problem of the grana can be interpreted differently if plastids have a fibrillar structure. This seems to follow from the properties of fragmenting carrot chromoplasts (Straus, 1950). According to these observations, the occurrence of "pigment needles" (Schimper, 1885; Courchet, 1888; Guillermond, 1917) was interpreted above as an expression of the fibrillar structure of the chromoplasts, not caused by crystallisation of the pigments. These fibrillae would be related, according to this view, to the "protein crystalloids" (needles) which Schimper found in several chromoplasts. They would be also related to "chondrioconts" and would produce carotinoid pigments as chondrioconts do (Guillermond, 1917, 1919). In living carrot cells, chromoplasts in the form of very thin and long fibrillae of reddish color can occasionally be found, as was indicated in chapter V.

Chloroplasts, too, seem to have a fibrillar structure, as observations by Frommann (1880), Schmitz (1884), Guillermond (1915), Mangenot (1920) and Emberger (1923) have indicated. The "rods of chlorophyll", seen by Courchet (1888) in the plastids of *Cucurbita Pepo*, and similar forms seen by Doutreligne (1935) in several chloroplasts, may also be part of this structure. Recently Lander (1935) observed a reticular structure in the plastids of *Anthoceros laevis* appearing like a group of parallel fine fibrils. According to Weier (1939), the fibrillar as well as the granular or homogeneous aspect of chloroplasts is physiological and can interchange in relation to cellular activity.

The structure of plastids would be better understood if the relationship between the fibrils (or lamellae) and the grana were known. According to Stewart (1948), fibrils connect the grana of the cloroplasts of *Isoetes* and form a network. This seems to correspond to the structure of carrot chromoplasts where sometimes

the grana could be seen lying in rows and in regular intervals on the fibrils (or lamellae) (Straus, 1950). The striated appearance of chloroplasts, observed by Heitz (1936) and Geitler (1937), also seems to indicate that the grana are arranged in rows and are connected by fibrils. Strugger (1950) saw the grana of pale green chloroplasts after vital staining with rhodamine B as little discs piled up one behind the other and he related the genetic properties of chloroplasts to the reduplication of the grana. Association of the grana with fibrils is assumed also by Doutreligne (1935) and by Weier and Stocking (1952). According to de Rezende-Pinto (1948), the grana of chloroplasts lie on fibrils, and the fibrils are arranged in a helical fashion. This observation is of great interest in view of the spiral forms of many chromoplasts of carrot. Thomas, Bustraan and Paris (1952) and Bustraan, Goedheer and Thomas (1952) have observed submicroscopic fibrillae in the stroma of spinach chloroplasts by electron microscopy. The authors compared these fibrils with the chromidia and interchromidia of cytoplasmic fibrils (Monné, 1948), as was done for the fibrils of carrot chromoplasts by Straus (1950). Thomas et al. did not relate the chromidia to the grana, as the present writer did. If grana and chromidia are related, this relationship would probably be genetic, since the grana and chromidia may differ in size and composition.

Above, the possible genetic relationship between chondrioconts and the fibrils of the chromoplasts was discussed, based on Guillermond's (1917, 1919) findings that carotinoid pigments are synthesised in chondrioconts. Since granules and rods (filaments) are the two most characteristic forms of mitochondria, it would be suggestive to relate also the grana to granular mitochondria (chondriosomes). The reversible association of the grana of chloroplasts into rodlets in vivo, observed by Doutreligne (1935), recalls indeed the transformation of chondriosomes into chondrioconts. If mitochondria would develop from submicroscopic constituents of the cytoplasm (microsomes or chromidia), as was discussed in chapter VIII, the genetic relationship between chromidia, mitochondria and grana would appear possible. In an important electron-microscopic study of mitochondria of various animal cells, Pallade (1952*b*) showed that these organelles contain a system of parallel, regularly spaced lamellae or ridges, 20–75 mμ wide, which protrude from the inside surface of the mitochondrial membrane

towards the interior. The orientation of the lamellae is more or less perpendicular to the long axis of the mitochondria. It is possible that the lamellae found by Pallade in mitochondria and the submicroscopic lamellae or fibrillae observed in chloroplasts and in carrot chromoplasts are related. If the homology of these formations should be demonstrated, the genetic relationship between mitochondria and plastids would be proved.

The structure of chromoplasts may be affected by variations in the proportion of stroma to pigments. Meyer (1883) and Schimper (1885) postulated a gradual disappearance of the stroma of chromoplasts during accumulation of the pigments. Carrot chromoplasts were considered as the extreme case where the stroma had completely regressed and only the pigments had remained. However, analysis of purified carrot chromoplasts has revealed a content of 20–40% carotene and 15–25% proteins (nucleoproteins), varying with the age of carrots and probably other factors (Straus, unpublished). In other chromoplasts the proportion of stroma to pigments may be quite different. It was observed by several investigators (Schimper, 1885; Courchet, 1888) that the pigments often appear in chromoplasts at first in the periphery. This may be related to the more pronounced organisation of the stroma in the periphery ("border fibers") as compared with the inner part. The proportion of stroma to pigments is perhaps different in the peripheral and inner portions of the chromoplasts.

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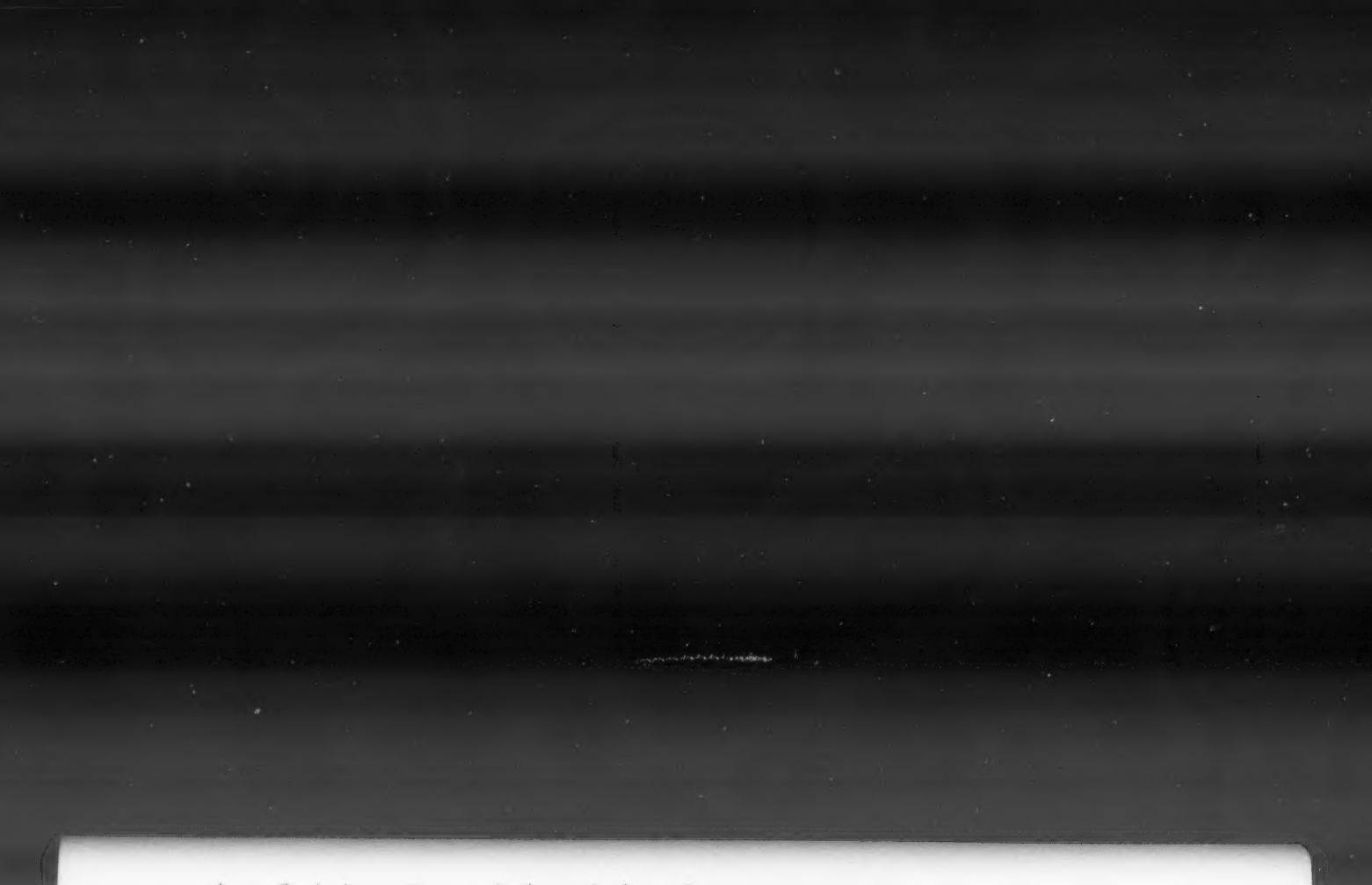

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